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Research article

EVALUATION OF ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA) FOR IgG IN THE DIAGNOSIS OF HUMAN HYDATID DISEASE.

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ABSTRACT: The aim of this study was to evaluate the role of ELISA for IgG antibodies against Echinococcus granulosus in detecting hydatid disease of liver and lung. The levels of IgG antibodies against Echinococcus granulosus were measured by indirect ELISA. A total of 32 patients were included in this study of which 20 had hydatid cysts in liver and 12 had in lung. Among 20 patients with hydatid cysts of liver, 18 had positive serology while 2 had negative serology. In patients having cysts in lung, 9 had positive serology while 3 had negative. The test showed 90% sensitivity in the diagnosis of hepatic cysts. However the sensitivity of this test was only 75% for pulmonary cysts. The overall diagnostic sensitivity of IgG ELISA in the diagnosis of both liver and lung hydatidosis was 84.37%. Conclusion: ELISA test is a sensitive test and can be used in the diagnosis of human hydatidosis.

Key words: Human hydatidosis, ELISA, IgG.

INTRODUCTION

Hydatid disease in man is caused principally by infection with the larval stage of the dog tapeworm Echinococcus granulosus. It is acquired by ingestion of eggs of the tapeworm which are excreted in the faeces of infected dogs. The larva of the cestode gets into portal system through intestines and lodge in various organs of the body. Liver is most common site followed by lungs, brain, muscle or any other body parts (Uma, et. al., 2005). Presentation to surgery is for alleviation of symptoms caused by pressure effects of the cyst and hence a definitive diagnosis is mandatory to prevent risks of rupture of the cyst. Clinical signs and symptoms guide the investigations. Since Echinococcus eggs are not shed by infected humans, serological determination is used for confirmation of the disease. Ultrasound scanning is widely available in India but it is only indicative of the disease and not diagnostic. CT scan and MRI may clinch diagnosis but are either too costly or not readily available hence serological testing is usually relied upon for confirmed diagnosis of hydatid disease. The serological tests in use include enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence antibody test, immunoelectrophoresis and immunoblotting. Among these, ELISA has been the technique which received most attention as an immunodiagnostic method for various parasitic infections. It is cheap and can be performed in poorly equipped laboratories (Zarzosa, et. al., 1999). The aim of the study was to assess the significance of IgG-ELISA in the diagnosis of human hydatid disease.

MATERIALS AND METHODS

Sample collection:

Blood samples collected from 5 healthy individuals and 32 surgically confirmed patients were centrifuged at 2000×g for 10 minutes at 4^0C to obtain serum. Haemolysed and lipaemic sera were excluded from this study. All serum samples were stored at -70^0C until antibody determination.

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Antigen preparation:

Fertile hydatid cysts were obtained from naturally infected sheep slaughtered at local abattoir. Hydatid fluid aspirated aseptically from the cyst was centrifuged at 2000×g for 20 minutes at 4^0C to remove the protoscoleces. The supernatant was passed through a Whatman membrane filter (WCN type cellulose nitrate, 47 mm diameter and 0.45 µm pore size). The fluid was then dialyzed against distilled water overnight at 4^oC using dialysis tubing (sigma Aldrich, USA) with molecular weight cut off 2000. Protein concentration of antigen was done as per the method of Lowry, et. al., (1951), with bovine serum albumin as reference standard.

Enzyme Linked Immunosorbent Assay (ELISA):

An indirect ELISA was performed by a modification of the method described by Wattal, et. al., (1986). The antigen was diluted to its optimum concentration (2µg/100µl) in carbonate buffer, pH 9.6; and100µl was placed in each well of a microtitration plate. After overnight incubation at 4^0C, the plate was washed four times with phosphate-buffered saline (PBS), pH **7.2,** containing Tween-20 0.05%. Test serum samples and control (Positive and Negative) sera (100µl of each) diluted 1 in 400 in PBS-Tween were added to each well. The plates were incubated for 1 hour at 37 ^{0}C temperature and washed with PBS-Tween. Goat anti-human IgG peroxidase conjugate (Sigma Aldrich, USA) was diluted to its optimum concentration (1 in 4000) in PBS-Tween and 100 µ1 was added to all wells of the microtitration plate. The plate was incubated for 1 h at 37 ^{0}C temperature, and then washed in PBS Tween. The substrate Tetramethylbenzedine (TMB) was used to visualize the antigen-antibody reaction. The reaction was stopped by the addition of 100 µ1 of 1M H_2S0_4 to all wells and the plate was read at 450nm on the ELISA reader. Cut off optical density (OD) for positive samples were taken as mean OD of 5 negative controls ±3 standard deviation.

RESULTS

The study was conducted on data over a period of 2 years. There were 10 males (6- hydatid of liver and 4- hydatid of lungs) and 22 females (14- hydatid of liver, 8- hydatid of lung) in the study group (Table 1). The median age was 35 years. Twenty patients (20) had cystic lesions in liver (14- right lobe, 3- left lobe and 3- both lobes). Twelve (12) patients had cystic lesions in the lungs (4 left lung and 8 right lung). All patients of hydatid liver and lung underwent ultrasonography and X- ray in abdomen and chest with reports suggestive of cystic lesions. Out of 20 patients of hydatid cysts of liver, 18 had a positive serology and 2 had negative serology. In patients having hydatid cysts in lung 9 had positive serology while the remaining 3 had negative serology. The sensitivity of the test for the diagnosis of hydatid liver was 90% while the sensitivity of the test for the diagnosis of hydatid lung was 75%. The overall sensitivity of ELISA in both groups of patients tested was 84.37% (Table 1). The diagnostic sensitivity of hepatic cysts was found greater than pulmonary cysts.

Table 1: Immunological profile of 32 surgically proved cases of hydatidosis by use of sheep hydatid antigen in the IgG-ELISA

Cyst Location	Male	Female	Total	IgG positive	Sensitivity
Liver	6	14	20	18	90%
Lung	4	8	12	09	75%
Total (n=32)	10	22	32	27	84.37%

DISCUSSION

The definitive diagnosis of human hydatid disease is by combination of Computed Tomography (CT) or Magnetic Resonance Imaging (MRI) with serological testing. Ultrasonography (USG) is indicative of the disease but not diagnostic. CT and MRI may not be easily available in remote areas and are costly too. In serology the detection of circulating Echinococcus granulosus antigens in sera is less sensitive than antibody detection, which remains the method of choice (Wenbao, et. al., 2003). Insensitive and nonspecific tests like Casoni's intradermal test, complement fixation test, indirect haemagglutination test and latex agglutination test have been replaced by ELISA, indirect immunofluorescence antibody test, immunoelectrophoresis (IEF) and immunoblotting (Lighowlers and Gottestine, 1995). Among the above ELISA for detection of IgG antibodies is the most commonly used test. It is considered to be highly sensitive and specific in detecting anti-*Echinococcus* antibodies (Wattal, et. al., 1986; Hanillo, et. al., 2005). The diagnostic sensitivity of IgG ELISA was found 90% in patients having cysts in liver while it was 75% in patients having cysts in lung. Our results are in the agreement with the results of Khuroo, (2002). He also reported that the diagnosis sensitivity of the ELISA is greater in patients having cysts in liver than the patients having cysts in lung. Gottstein, (1992) reported the similar findings that hepatic cysts are diagnostically sensitive enough as compared to cysts in lung. In case of hydatid cyst of liver the sensitivity of the test was 90% which is in confirmation with Poretti *et al*., (1999); Nasrieh and Abdel-Hafiz, (2004); Hanillo, et. al., (2005). Pulmonary hydatidosis is associated with poor seropositivity than liver hydatidosis, attributed to production of low levels of antibodies in these patients (Babba, et. al., 1994). Similar results were also associated in our study.

Conclusion: IgG-ELISA test is the most commonly employed test for the diagnosis of the hydatid disease. The test has a very high diagnostic sensitivity as compared to other tests like Casoni's intradermal test, indirect heamagglutination test and complement fixation test. Due to its high sensitivity and low cost it can be employed in the diagnosis of human hydatidosis.

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